



Antibacterial Activity of Ethyl Acetate Fraction of Passion Fruit Peel (*Passiflora edulis* Sims) on *Staphylococcus Aureus* and *Escherichia Coli*

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Abstract. North Sumatera is one of the central areas of purple passion fruit production in Indonesia. Processing passion fruit into beverage products (passion fruit juice) produces peel has not been utilized. The use of passion fruit skin needs to be studied so that it can be useful as a raw material for antibacterial drug preparations. The aim of this study was to determine the phytochemical constituent screening and antibacterial activity of ethyl acetate fraction of purple passion fruit peel against *Staphylococcus aureus* and *Escherichia coli*. Simplicia and ethyl acetate fraction were determined its phytochemical properties. The extraction process by percolation method using ethanol 96% and continue to fractionation process by liquid liquid extraction method using n-hexane and ethyl acetate. The antibacterial activity were tested against *Staphylococcus aureus* and *Escherichia coli* using agar diffusion method with paper discs. The result showed that the simplicia characteristic of passion fruit peel were water content of 8.64%, water soluble extract of 31.69%, ethanol soluble extract of 13.02%, ash total of 7.89%, and insoluble ash in acid of 0.816%. The phytochemical screening simplicia and ethyl acetate fraction showed the presence of flavonoids, glycosides, saponins and tannins. The antibacterial activity test showed that the ethyl acetate fraction has an effective inhibition at the concentration of 100 mg/ml against *Staphylococcus aureus* and *Escherichia coli*, it showed dose dependent manner. The ethyl acetate fraction of passion fruit peel (*Passiflora edulis* Sims) has an antibacterial activity on *Staphylococcus aureus* and *Escherichia coli*

Keywords: *Passiflora edulis* Sims, ethyl acetate fraction, antibacterial, *Staphylococcus aureus* and *Escherichia coli*

Abstrak. Sumatera Utara adalah salah satu daerah pusat produksi buah markisa ungu di Indonesia. Pengolahan buah markisa menjadi produk minuman menghasilkan limbah kulit yang belum dimanfaatkan. Manfaat kulit buah markisa dikembangkan sehingga dapat bermanfaat salah satunya yaitu sebagai antibakteri. Tujuan dari penelitian ini adalah untuk menentukan kandungan fitokimia dan aktivitas antibakteri fraksi etil asetat kulit buah markisa ungu terhadap *Staphylococcus aureus* dan *Escherichia coli*. Siplisia dan fraksi etil asetat ditentukan sifat fitokimia. Proses ekstraksi dengan metode perkolasi menggunakan etanol 96% dan berlanjut ke proses fraksinasi dengan metode ekstraksi cair menggunakan n-heksan dan etil asetat. Aktivitas antibakteri diuji terhadap *Staphylococcus aureus* dan *Escherichia coli* menggunakan metode difusi agar dengan cakram kertas. Skrining fitokimia simplisia dan fraksi etil asetat menunjukkan adanya flavonoid, glikosida, saponin, dan tanin. Uji aktivitas antibakteri menunjukkan bahwa fraksi etil asetat memiliki penghambatan

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efektif pada konsentrasi 100 mg / ml terhadap *Staphylococcus aureus* dan *Escherichia coli*. Fraksi etil asetat kulit buah markisa (*Passiflora edulis* Sims) memiliki aktivitas antibakteri pada *Staphylococcus aureus* dan *Escherichia coli*.

Kata kunci: *Passiflora edulis* Sims, Fraksi etil asetat , antibakteri, *Staphylococcus aureus* and *Escherichia coli*

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1. Introduction

Purple passion fruit (*Passiflora edulis* Sims) contains some phytochemical constituent that has potential effect as antibacterial. Leaves of purple passion fruit contain some secondary metabolite such as glycosides, tannins, flavonoids, saponins and alkaloids, where as the stems contain of glycosides, flavonoids, saponins and alkaloids, while fruits contain glycosides, tannins, flavonoids and alkaloids [1]. The latest study reported that methanol extract of purple passion fruit leaves had antibacterial activity against several bacteria such as *Staphylococcus aureus*, *Staphylococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* and *Salmonella typhi* [2].

Purple passion fruit is planted in tropical highlands and subtropical areas around of 700 to 2000 m above sea level with temperatures of 18 to 25°C. The central production of passion fruit in Indonesia was North Sumatera province. The passion fruit was processing into beverage product that produced passion fruit peel waste. The phytochemical constituent of passion fruit peel has potential effect as a sources of medicine which is need to be examined such as its antibacterial effect. It is necessary to determine antibacterial activity of ethyl acetate fraction of purple passion fruit peel on *Staphylococcus aureus* dan *Escherichia coli*.

2. Materials and Methods

The materials that used in this study were purple passion fruit peel, nutrient agar, nutrient broth, distilled water, ethanol, ethyl acetate, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. Fresh fruit of *Passiflora edulis* Sims were collected in Central Market of Medan, Indonesia. The sample was identified at Indonesian Institute of Science, Research Center of Biology, Bogor, Indonesia. Authentication number 440/IPH.1.01/lf.07/IV/2015

2.1 Extraction

An amount of 300 g dried material plant samples were crushed in a blender, then macerated in ethanol 96 % for 3 hours then moved to perlocator tube. Percolation process was stopped if the last 500 mg of solvent were evaporated, and leaving no residuals. The solvent was evaporated at low pressure with a temperature of not more than 40 ° C using a Rotary evaporator. The crude

extract were continued to fractionation process by liquid liquid extraction method using n-hexane and ethyl acetate

2.2 Phytochemical Screening

Phytochemical simplicia and ethyl acetate fraction of passion fruit peel includes examining the chemical secondary metabolites of alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids [3-5].

2.4 Antibacterial Activity of ethyl acetate fraction of purple passion fruit

Bacterial colonies were taken from the culture stock with a sterile ose needle then suspended in a test tube containing 10 ml of broth nutrient media, then measured turbidity of the solution at a wavelength of 580 nm until a 25% transmittance was obtained which was equivalent to 10⁶ CFU (Colony Forming Units) [6].

As much as 1 g crude extract of ethyl acetate fraction of passion fruit peel was carefully weighed on an analytical balance. Extract was dissolved in 2 ml DMSO solvent in a vial and obtained extract concentration of 500 mg / ml. The solvent was diluted to several concentrations of 400 mg / ml, 300 mg / ml, 200 mg / ml, 100 mg / ml, 75 mg / ml, 50 mg / ml, 25 mg / ml, 12.5 mg / ml and 6.25 mg / ml. The bacteria inoculum as many as 0.1 ml is put into a petri dish, then added 20 ml of nutrient agar wait until the temperature reaches 45°C, homogenized and left it until the media become a solid form. Furthermore, paper discs (diameter of 6 mm) are immersed in extract solution on various concentrations, dried and placed on the surface of the agar media. Media was incubated at 36-37 ° C for 18-24 hours. The diameter of the inhibitory area around the paper disc is measured using a calipse. The test was carried out 3 times [6].

3. Result and Discussion

The qualitative test of phytochemical properties on simplicia, ethanol extract and ethyl acetate fraction purple passion fruit shows in table 1.

Table 1. Phytochemical screening of simplicia, ethanol extract and ethyl acetate fraction of purple passion fruit

| No. | Phytochemical properties | Simplicia | Ethanol extract | Ethyl acetate fraction |
|-----|--------------------------|-----------|-----------------|------------------------|
| 1. | Alkaloids | - | - | - |
| 2. | Flavonoids | + | + | + |
| 3. | Glycosides | + | + | + |
| 4. | Anthraquinone glycosides | - | - | - |
| 5. | Saponin | + | + | + |
| 6. | Tanin | + | + | + |
| 7. | Steroids/Triterpenoids | + | + | - |

Table 1 shows that the simplicia and ethanol extract purple passion fruit have several compound such as flavonoids, glycosides, anthraquinone glycosides, saponin, tanin and steroids/triterpenoids. Ethyl acetate fraction contain of flavonoids, glycosides, anthraquinone glycosides, saponin and tanin. Flavonoids, tanin, saponins and steroids / triterpenoids are phytochemical compounds that have potential effect as antibacterial and antiviral agents.

The results of antibacterial test of ethyl acetate fraction on *Staphylococcus aureus* and *Escherichia coli* can be seen in the table 2.

Table 2. Diameter of Inhibiting area on *Staphylococcus aureus* and *Escherichia coli*

| No. | Concentration (mg/ml) | Diameter of Inhibition area (mm) | | | | | | | |
|-----|-----------------------|----------------------------------|------|------|-------|-------------------------|------|------|-------|
| | | <i>Staphylococcus aureus</i> | | | | <i>Escherichia coli</i> | | | |
| | | D1 | D2 | D3 | Mean | D1 | D2 | D3 | Mean |
| 1. | 500 | 20.7 | 20.4 | 20.3 | 20.46 | 20.2 | 20.5 | 20.6 | 20.43 |
| 2. | 400 | 19.3 | 19.2 | 19.4 | 19.3 | 19.4 | 19.7 | 19.5 | 19.53 |
| 3. | 300 | 18.2 | 18.2 | 18.3 | 18.23 | 18.3 | 18.6 | 18.4 | 18.43 |
| 4. | 200 | 16.3 | 16.4 | 16.5 | 16.4 | 16.7 | 17.1 | 16.8 | 16.86 |
| 5. | 100 | 14.2 | 14.3 | 14.1 | 14.2 | 14.2 | 14.3 | 14.2 | 14.23 |
| 6. | 75 | 12.5 | 12.1 | 12.4 | 12.33 | 12.5 | 12.4 | 12.2 | 12.36 |
| 7. | 50 | 11.6 | 11.3 | 11.4 | 11.43 | 10.9 | 11.2 | 11.0 | 11.03 |
| 8. | 25 | 10.4 | 10.2 | 10.3 | 10.3 | 9.6 | 9.5 | 9.4 | 9.5 |
| 9. | 12.5 | 7.5 | 7.2 | 7.3 | 7.33 | 7.2 | 7.4 | 7.2 | 7.26 |
| 10. | 6.25 | - | - | - | - | - | - | - | - |
| 11. | Blank | - | - | - | - | - | - | - | - |

where:

D : diameter (mm)

The concentration of extract which fulfill the requirements of Indonesian Pharmacopoeia was the concentration of extracts with an effective inhibition area of approximately 14-16 mm [6]. The results of antibacterial activity of ethyl acetate fraction on *Staphylococcus aureus* and *Escherichia coli* showed an effective inhibition at the concentration of 100 mg / ml with an effective inhibition diameters were 14.2 mm and 14.23 mm respectively. Minimum inhibition concentration of ethyl acetate fraction against *Staphylococcus aureus* and *Escherichia coli* at the concentration of 12.5 mg / ml with inhibitory diameter of 7.33 mm and 7.26 mm respectively. Ethyl acetate fraction of passion fruit peel has an effect in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria due to its phytochemical properties that has strong antibacterial activity, namely flavonoids, tannins and saponins.

Tannins are phenol compounds that are widely distributed in vascular plants. Phenol compounds and their derivatives were one of the antibacterials that works by disrupting the function of the cytoplasmic membrane, low concentrations of phenol compound could damage the cytoplasmic membrane which causes leaking of metabolites that is important in bacterial enzyme system, while at high concentrations, it could damage the cytoplasmic membrane and proteins of cell [5,

7]. Saponins are used as antimicrobials in recent years. The mechanism of action of saponins as antibacterials is to reduce surface tension resulting in increased permeability or leakage of cells, it resulting discharge of intracellular compounds [8]. Steroids and triterpenoid also has antibacterial activities, some studies reported antibacterial activity of steroid and triterpenoid against several bacteria [9-11].

The results showed that the *Staphylococcus aureus* bacteria had a greater inhibitory zone compared to *Escherichia coli* bacteria in various concentrations of the test solution. The difference occurs because the two test bacteria species have different composition and cell wall structure. Gram-positive bacteria more susceptible to chemical compounds than gram-negative. The cell wall structure of gram-positive bacteria is simpler, it has single-layered with a low lipid content (1-4%) making it easier for bioactive ingredients to enter the cell. The structure of cell wall of the gram-negative bacterial is more complex, it has three-layered consisting of the outer layer of lipoprotein, the middle layer of lipopolysaccharide which acts as a barrier to antibacterial bioactive material, and an inner layer of peptidoglycan with high lipid content (11-12%) [12-14].

4. Conclusion

Antibacterial activity of ethyl acetate fraction of purple passion fruit on *Staphylococcus aureus* and *Escherichia coli* showed an effective inhibition at the concentration of 100 mg / ml, it is showed dose dependent manner.

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